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A Histological Evaluation of Experimentally Produced Periapical Pathosis in the Adult Beagle Dog

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A HISTOLOGICAL EVALUATION OF EXPERIMENTALLY PRODUCED
PERIAPICAL PATHOSIS IN THE ADULT BEAGLE DOG

By
Ronald/Brown, D.D.S.

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

May

1984

DEDICATION

To my wife, Rita, whose love and patience
allowed me to pursue this educational
endeavor so late in life.

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To Dr. Franklin Weine, outstanding teacher and clinician, and most of all good friend, I extend my most sincere gratitude and appreciation for guiding me through this research project as my committee chairman and providing me with an outstanding endodontic education as my teacher.

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VITA

The author, Ronald Brown was born in New York, New York on August 13, 1932.

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In August 1982, he entered Loyola University School of Dentistry in a dual course of study leading to the degree of Master of Science in Oral Biology and a Certificate of Specialty Training in Endodontics.

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CHAPTER I

INTRODUCTION

Animal models are usually used in pulpal/periapical studies due to the difficulty in obtaining suitable material from humans and the recent restrictions on the use of humans in experiments. The use of primates is a desirable alternative but the high cost of these animals has precluded their use in many investigations. The dog has been utilized frequently as an animal model in endodontic research and has been found to be very useful.¹⁻⁵ The beagle dog was chosen for this study because of its availability, reasonable cost, and ease of maintenance.

Some investigations require the creation of periapical pathosis early in the research in order to evaluate the effects of various endodontic treatment procedures being studied.

The purpose of this study is to standardize a regimen to create periapical pathosis in adult beagle dogs.

The importance of bacteria in the development of periapical pathosis has been demonstrated by the experiments of Kakehashi, Stanley, and Fitzgerald.⁶ They showed that the exposed pulps of animals raised in germ-free environments degenerated and led to periapical pathosis only when bacteria were introduced.

Streptococci are the most frequently isolated microorganisms from infected root canals.^{7,8} This regimen will employ a specific number of the microorganism Streptococcus faecalis as the pathogenic agent. S. faecalis has been implicated as a significant endodontic pathogen.^{7,8}

CHAPTER II

REVIEW OF THE LITERATURE

THE DOG IN ENDODONTIC RESEARCH

Hill³ studied experimentally produced dental granulomas in dogs. He found that in their morphologic and histologic aspects, experimental dental granulomas in dogs were comparable to human dental granulomas.

Dixon and Rickert⁹ stated that there was no definite agreement among research workers as to which research animals would give similar histologic reactions to those found in the human. They selected the dog as their experimental animal because the dog is an omnivorous mammal and is biologically similar to man, as could be demonstrated from their findings of the characteristics of the inflammatory reactions in histologic sections. They found that the transition from an active purulent process to a chronic exudative and proliferative process, as evidenced by a marked increase in lymphocytes and plasma cells and an occasional remaining polymorphonuclear leukocyte, was the same in the dog as in humans.

Pisanti and Sciaky¹⁰ used a two-year-old male dog in their investigation showing that calcium from calcium hydroxide dressing did not contribute to the calcium in newly formed dentin in pulpally exposed teeth.

Seltzer, Turkenkopf, Vito, Green, and Bender,¹¹ used 64 teeth from three dogs to examine histologically as well as roentgenographically the periapical tissues of endodontically treated teeth six to twelve months

after completion of treatment. Observations of the periapical tissue reactions around sterile and non-sterile canals were compared. They stated that although human material would have been more desirable, the experiments were performed on dogs because control of experimental conditions could be more precise. Also they stated that it was difficult to subject humans to block resections which were necessary for obtaining the tissue needed in the study of periapical reactions.

Synder, Seltzer, and Moodnik¹² employed three adult male Collie dogs to study the effects of N2 in experimental endodontic therapy. In an effort to simulate clinical conditions in human teeth, dental pulps in dog teeth were injured and exposed to the oral fluids for varying periods of time to produce inflamed or necrotic pulps. In one of the dogs the pulps were not injured prior to the experimental procedures. Root canal procedures comparing the N2 method with standard endodontic techniques were performed and comparative histological studies of the periapical tissue reactions were made.

Matsumiya and Kitamura¹³ evaluated four types of topical disinfectants and calcium hydroxide paste in the sterilization of filling of 215 infected root canals which had been experimentally created in the teeth of 17 dogs. The relation of the effects of sterilization in the root canals and healing conditions in the periapical tissues was histopathologically and histobacteriologically studied.

Torneck and Tulananda¹⁴ performed 12 pulpotomies in a one and one half-year old 15 lb. female dog. They concluded that removal of the dental pulp in the dog and exposure of the root canal to the oral

environment could result in the establishment of a periapical abscess. Where this abscess was associated with a substantial degree of periapical bone resorption and loss of periapical support, it could induce changes in the remaining portion of the periodontal ligament which were associated with new bone formation along the osseous border of the periodontal space. This effect might be related to stimulative or compensative factors. Also induced were changes in the pattern and distribution of the periapical trabeculae. These changes were not static but dynamic, varying with changes in the local and possibly systemic environment. Finally, they stated that the entire pattern of change was possibly representative of dogs, and in the absence of more specific material, direct correlation with humans could not be made.

Bhaskar and Rappaport⁵ conducted a study using 39 teeth from two dogs. They evaluated the effects of overfilling and underfilling root canals with silver cones and gutta percha cones. They also studied healing of perforated furcations and debrided but unfilled root canals where only the pulp chambers were sealed with occlusal fillings.

Davis, Joseph and Bucher¹⁵ treated 32 root canals in four mongrel dogs. The mandibular third and fourth premolars were selected because their apices were widely spaced and could be studied individually without danger of confusion. Fourteen canals were underfilled by 3 mm, nine canals were filled to within 1 mm of the roentgenographic apex, and nine canals were overfilled. The periapical reaction to these various filling techniques was studied.

Barker and Lockett¹ wrote about the utilization of the mandibular

premolars of the dog for endodontic research. They recommended using the 2nd, 3rd, and 4th mandibular premolar teeth in large dogs such as Alsatians for experiments involving pulp capping or root canal procedures. They described the mandibular 2nd premolar of the dog as usually having a single root but occasionally having two roots, like the 3rd and 4th premolars. Furthermore, they noted that the canals of dog premolars were sufficiently wide to allow instrumentation to the apical region with conventional equipment. Also they stated that the crowns were blade-like and the pulp chamber passed from a prominent central horn to smaller mesial and distal horns before entering the root canal and therefore it was possible to open each canal individually so that different root canal treatments might be carried out in the mesial and distal roots of a single tooth for the purpose of subsequent comparisons of periapical response. Since the mandibular premolars were not in contact with the maxillary teeth, any apical periodontitis which may supervene following operative procedures were not complicated by occlusal stress. They also described the pulp tissue of the dog as being morphologically similar to human pulp, but as appearing to be more prone than human pulp to degeneration following capping experiments. Discussing the anatomy of dog root apex they noted that at the cemental dentinal junction, which was situated at some distance from the actual apex, the main canal broke up into a complexity of fine channels which radiated peripherally, and were all confined to the cementum. They said that although such complexity was not seen in apices of human teeth, apical ramifications did occur, and the main canal often pursued a tortuous course to its termination.

Binnie and Rowe¹⁶ examined histologically the apical periodontal tissues of 192 premolar teeth of eight six month-old beagle dogs to record the incidence of epithelial cell rests, proliferations, and cysts. These teeth, which had incompletely formed roots, had been treated with standardized endodontic procedures in order to examine the tissue effects of various root filling materials.

Isermann and Kaminsky¹⁷ used 32 teeth from eight month-old beagle dogs to evaluate the influence of Dycal as a direct pulp capping agent on exposed and bacterially infected teeth. The effects were evaluated with the use of serial radiographs, consecutive vital dye injections, and histological sections. In another study¹⁸ they reported on the pulpal responses to bacterial infection in the dog. They used 26 teeth from eight month-old beagle dogs. Eight of the teeth were bacterially infected and exposed. Ten of the teeth were bacterially infected but not exposed. The remaining teeth were operated and unoperated controls. They stated that the bacterial effects upon the human pulp were known, but in experimental animals the response to bacteria did not appear to be uniform. The results of their study, however, demonstrated a close correlation between dog and man with respect to bacterial penetration of dentinal tubules and the pulpal inflammation associated with bacteria and their metabolic products.

Jew, et al.,¹⁹ used six adult beagle dogs to investigate the histologic response of the periodontium to nonsurgical repair of endodontic perforations sealed with Cavit. A total of 36 lower premolar teeth were used in the study. The teeth were treated endodontically and the canals

were filled with gutta percha and sealer. Immediately thereafter 31 experimental perforations were created and filled with Cavit. An additional seventeen perforations were created and left unfilled to serve as controls.

McCormick, Weine, and Maggio²⁰ studied tissue pH of developing periapical lesions in six beagle dogs age five months. The pulps of 24 premolar teeth were extirpated and inoculated with a pure culture of S. faecalis. Untreated premolar teeth adjacent to the treated teeth served as controls. One week later, the root canals of two-thirds of the experimental teeth received endodontic canal preparation, and then either an acid (pH = 3), or a basic (pH = 11) paste was inserted. These teeth served as either acid-treated or basic-treated teeth, whereas the remaining experimental teeth served as infected only teeth. The periapical tissue pH in the region of the apices was measured at various time intervals using pH microelectrodes.

THE ROLE OF MICROORGANISMS IN PERIAPICAL DISEASE

Kakehashi, Stanley, and Fitzgerald⁶ conducted a study using germ-free animals to test the influence of viable microorganisms on the fate of the surgically exposed dental pulp. They observed the pathologic changes resulting from untreated experimental pulp exposures in germ-free rats as compared with conventional rats with a normal complex microflora. The pulp tissues of these rats was exposed by drilling through the occlusal surface of the maxillary right first molar with a round carbide bur mounted in a jeweler's spindle-topped hand mandrel. After varying

post-operative time intervals (1 to 42 days), the animals were killed and the appropriate tissues were serially sectioned. By the eighth day vital pulp tissue remained only in the apical half of the roots of the conventional animals. Complete pulp necrosis with granulomas and abscess formation occurred in all older specimens. Evidence of repair was uniformly lacking. In contrast, no devitalized pulps, apical granulomas, or abscesses were found in the germ-free animals. Dentinal bridging began at 14 days, and by 21 and 28 days was complete, regardless of the angle or severity of the exposure. The results, even in the face of gross food impaction, indicated that the presence or absence of a microbial flora was the major determinant in the healing of exposed rodent roots.

Morse²¹ described several pathways by which microbes and microbial products may reach the pulp:

1. through a carious lesion either directly or via dentinal tubules
2. through a cavity preparation, either when the pulp is directly exposed or via dentinal tubules
3. as a result of periodontal disease from pocket or furca involvement via lateral canals or dentinal tubules, from apical blood vessels or lymphatics by apical extension of a periodontal pocket
4. through enamel lamellae or dead tracts in dentin
5. from an adjoining periapical lesion via apical or lateral canals
6. as a result of anachoresis
7. from heat and pressure through dentinal tubules
8. abrasion, erosion, attrition, fracture, or developmental anomaly

Blechman²² wrote "whereas the presence of microorganisms does not prove infection, their ability to invade and multiply in the tissues of the host does."

Naidorf²³ described a necrotic pulp as accompanied by a cessation of blood circulation in the canal resulting in a "privileged sanctuary" from which bacteria, toxins, and protein degradation products could emanate and which was itself insulated from the normal clearing process and healing mechanisms of the body. Also he stated "once the organisms have gained a foothold in the pulp and have begun to multiply, the possibility of a spread to adjacent periapical tissues is a result of two opposing forces, namely the virulence of the organisms and the resistance of the host."

The pulpal disease state due to microorganisms has been expressed in the following basic formula by Hobson.²⁴

$$\text{Pulpal Disease State} = \frac{\text{number of microorganisms} \times \text{virulence}}{\text{resistance of host}}$$

Torneck² described the way in which infection of the pulp and periapical tissue occurred. He proposed the following hypothesis: The process of periapical disease may be initiated by an injury to the dental pulp or by an invasion of the injured pulp tissue with microorganisms from the oral cavity. This action may in turn lead to the establishment of an inflammation but, as yet, not an infection of the underlying periapical tissues, for these tissues normally possess an effective defense mechanism for resisting microbial invasion and localizing it to the confines of the root canal. At this point the role of microorganisms are

unable to infect directly the surrounding tissue, although they are about to initiate some reaction to them. However, if the situation is altered by the superimposition of a systemic disturbance, a predisposing local injury, a local increase in the growth of certain virulent microorganisms, or a symbiotic effect of one group of microorganisms with another, there could be an invasion of the periodontium, followed quickly by the onset of periapical infection.

Smith, Thomassen and Sweet²⁵ described the isolation of a number of microorganisms from diseased pulp canals and the production by these microorganisms of various enzymes which have been associated with pathogenicity and/or invasiveness. The root canal material was obtained aseptically from 90 teeth with diseases of the pulp but no direct exposure to the oral cavity. Ninety-five strains of organisms were obtained in pure culture. Fifteen produced alpha hemolysin, 36 produced beta hemolysin, 29 produced fibrolysin, 4 produced coagulase, 20 produced hyaluronidase and 12 produced proteolytic enzymes.

Grossman and Oliet²⁶ studied the correlation of the bacteriologic states of the coronal and radicular portion of the pulp. They found that agreement between the culture of the coronal pulp and that of root canal occurred in 77 percent of the cases and disagreement occurred in 23 percent. Where the latter occurred, the radicular pulp was positive 14 percent more often than the coronal pulp.

Korzen, Krakow, and Green²⁷ used conventional and monoinfected gnatobiotic rats in their study. They concluded that the severity of the inflammatory response in pulpal and periapical tissues was related

to the quantity of microorganisms present within the root canal and the length of time the tissues were exposed to microorganisms.

Kennedy, Hamilton and Syverton²⁸ reported on bacteriologic and histopathologic studies of the effects of inoculation of streptococci into the root canals of eight monkeys. They found that experimental root canal lesions were revealed by histopathologic examination but only rarely by radiologic examination.

Bartels, Naidorf, and Blechman²⁹ conducted a study to determine the causative factors of "flare-ups" which occurred during endodontic therapy. Smears and cultures from such cases were collected. They found that members of the streptococci family were found most frequently.

Winkler and Van Amerongen⁷ reported on the bacteriological results of more than 4000 cultures from more than 1000 canals. They found that streptococci form 61 percent of the isolated organisms and seemed to be the most serious pathogens. They also found that S. faecalis were very persistent and difficult to eliminate from root canals as seen by their high frequency in subsequent cultures.

S. faecalis was the enterococci most frequently isolated in human saliva. It was found in approximately 20 percent of saliva samples.³⁰

Engstrom³¹ investigated certain problems arising in connection with enterococci infections in the tooth pulp. He found that there was a direct correlation between the occurrence of enterococci in the oral cavity and in the pulp cavity. He also found that enterococci infection of the pulp cavity posed a treatment problem, as they were difficult to eliminate and caused the treatment period to be greatly prolonged.

Crawford and Shankle³² examined the flora of open and closed teeth and found that non-hemolytic streptococci predominated.

Heintz, Deblinger and Oliet³³ took cultures from debrided and medicated root canals and screened them for enterococci. Forty-four percent contained S. faecalis or one of its varieties. They noted that the ability of enterococci to persist after root canal treatment, their known resistance to antibiotics and disinfectants, and their ability to survive in adverse conditions indicated they could be a cause of post operative infection.

Weine³⁴ described S. faecalis as being pathogenic but of low virulence. He stated that they could be difficult to eliminate from the root canal.

Bender, Seltzer, and Kaufman³⁵ used S. faecalis in a study to see (1) whether bacteria present in deeply cut dentin could penetrate into the pulp, (2) whether the use of pressure would increase bacterial penetration and (3) whether a sterilizing agent could prevent the passage of live bacteria through the dentinal tubules.

Hedman³⁶ used cannulas and culture wires that passed through the cannulas into periapical lesions. He found that in pulp involved teeth with radiolucent areas, 68.5 percent had bacteria in both the pulp canal and periapical region before debridement of the root canal. Also he found that when streptococci was present in the pulp canal it was also present in the periapical tissue.

Block, Bushell, Rodriques, and Langland³⁷ found, however, that whole bacterial cells were present in only 23 of 230 periapical endodontic

surgical specimens.

Grossman³⁸ investigated the minimum number of bacteria needed to initiate growth in a culture media. He found that although there was variation among organisms and media, only one organism of a certain bacteria was sufficient to cause growth and multiplication in a suitable media.

Palmer, Lazzaratto and Weine³⁹ used a specifically traceable strain of S. faecalis that was serially diluted to inoculate prepared root canals. The number of organisms in each dilution was determined by counting colonies on pour plates. When they cultured these canals they found that fewer than ten organisms gave positive readings in Thioglycollate media when the media was incubated for seven days.

PULPOPERIAPICAL PATHOSIS

Simon⁴⁰ described periapical inflammation as an extension of the pulpal inflammatory response, the basic microvascular activities of the periodontal ligament being similar to those which occurred in the pulp. The periodontal ligament unlike the pulp, however, has a rich collateral circulation that greatly enhances the ability of the periapical tissues to respond to inflammation.

Langeland, Block, and Grossman⁴¹ found acute and chronic inflammatory cells present in all of 35 endodontic surgical specimens. The types of chronic cells found were lymphocytes, monocytes, plasma cells, macrophages, foam cells, mast cells, and foreign body cells. The cells representing acute inflammation were neutrophilic leukocytes.

Morse⁴² proposed that certain immunological mechanisms are responsible for the responses to intracanal and periapical irritants in the periapical area. These irritants, which are antigenic, become localized in the periapical area because of the dense wall of alveolar bone. The immune responses that could result may consist of any or all of these components - arthus phenomena, immediate hypersensitivity, cytotoxicity, or delayed hypersensitivity. Also, as macrophages breakdown, bacterial products may be released that could stimulate B and T lymphocytes and result in specific antibodies or sensitized T lymphocytes.

Torabinejad and Kettering⁴³ reported that antigens entering the periapical tissues from a root canal with pulpal disease could include viable or dead bacteria, bacterial products, and denatured host tissue subsequent to pulpal deterioration. They also suggested that antigen-antibody or immune-complex reactions played a role in the pathogenesis of periapical disease.

Goaz and Wood⁴⁴ described the generation of pulpoperiapical pathosis as being a consequence of pulpitis with or without pulp death and that one or a combination of sequelae could be expected at the periapex. First, irritating products could arrive at the periapex but in such moderate amounts that the host defenses would be able to combat and localize their effects. The resultant inflammation could be of a chronic nature and a periapical granuloma would result. Second, in teeth with gangrenous pulps, the number and virulence of the bacteria from the infected root canal could be sufficient to overwhelm the defenses of the periapical

tissues and consequently an acute periapical abscess would develop. If the host defenses partially control the infection, a chronic periapical abscess would result. The dental granuloma could in turn evolve into one of several entities depending on the interaction of certain factors. If the epithelial rests of Malassez proliferate and undergo intraepithelial degeneration, a radicular cyst could result. If infection of the cyst or granuloma occurs an acute periapical abscess could occur.

Smulson, Maggio, and Hagen^{4,5} described the development of the periapical granuloma as follows. The presence of necrotic pulp tissue in the root canal (zone of necrosis) results in diffusion of the toxic material into and slightly beyond the area of coalescence of the pulpal and periapical connective tissue. Without radiographic evidence centers of cellular infiltration will appear around each foraminal opening. Capillary dilation occurs and leukocytes are attracted to the area. Closest to the zone of necrosis (zone I) are the neutrophilic leukocytes. These are surrounded by large masses of lymphocytes and plasma cells. The initial mild chronic response increases as more of the necrotic products and microorganisms, if present, diffuse from the pulp canal into the periapex. The toxicity of the root canal irritant is reduced by the fluid and cellular exudative activity in the zone of contamination (zone II). This reduction in toxicity stimulates cells to join together to form multinucleated osteoclasts, which resorb the contaminated periapical bone. The gap that is opened in the bone surrounding the lesion will ultimately be filled with granulomatous tissue to form zone III, the zone of irritation. At the periphery of the granulomatous zone the toxicity of the root canal

irritants becomes so diminished that the irritants act as a stimulus to fibroblasts and osteoblasts forming a fourth and outer zone, the zone of stimulation.

Shear⁴⁶ discussed the histogenesis of the dental cyst and said that the most widely held view was that the epithelium within a periapical granuloma underwent proliferation in response to the stimulus provided by an inflammatory process in the vicinity.

Seltzer, Soltanoff, and Bender⁴⁷ confirmed in their investigation that cell rests of Malassez in the region of the apical foramin can proliferate in the presence of periapical inflammation, possibly with the eventual formation of a periapical cyst.

Binnie and Rowe,¹⁶ however, concluded from their study on dogs that the presence of epithelial rests and hence proliferations and cysts appeared to be characteristic of the individual animal. Also they found that epithelial proliferation would not appear to occur in the presence of acute inflammation.

There are two schools of thought as to how apical cysts develop. One school of thought suggests that in an area of chronic inflammation a connective tissue cavity is created when cells break down and become necrotic. The epithelial cells may proliferate and line this cavity to form a cyst.⁴⁰ On the other hand, Ten Cate⁴⁸ believes that the cyst may arise by intraepithelial degeneration and autolysis of central cells within the proliferating epithelial rest cells.

Bhaskar⁴⁹ examined 2,308 cases that involved radiolucencies at the apical areas of teeth. Microscopically, these apical specimens represented

nine different types of lesions: (1) dental granuloma, (2) radicular cyst, (3) residual cyst (4) apical scar, (5) cementoma (stage I), (6) dental abscess, (7) foreign-body reaction, (8) cholesteatoma, and (9) giant-cell lesion.

Patterson and Hillis⁵⁰ found that scar tissue was occasionally found at the apex of pulpless teeth. They thought that the formation of the scar tissue was the result of a very mild infection in the pulp canal.

Simon⁵¹ described periapical pulpo-osteosclerosis as a radiopaque area occurring around the periapex of a tooth with pulp disease. Another term for this type of pathosis is condensing osteitis. The pulp of the involved tooth may be vital, partially necrotic, or completely necrotic. This increase in bone density that is roentgenographically visible is a result of localized increased bone formation and is an osseous reaction to a low-grade irritant.

Shafer, Hine, and Levy⁵² wrote that it is important to realize that periapical lesions do not represent individual and distinct entities, but rather that there is a subtle transformation from one type of lesion into another type in most cases. Furthermore, it should be appreciated that a certain type of reversibility is possible in some lesions.

The frequency of occurrence of various pulpoperiapical lesions reported varies markedly among investigators. This disagreement may be attributed to many factors such as definition of a cyst, histologic criteria, sample size of the population, and unique characteristics peculiar to each population sample.⁴⁰

Wais⁵³ conducted a study of periapical lesions from 100 nonvital anterior teeth. The periapical tissues were examined microscopically by an oral pathologist. The study was divided into two parts of 50 teeth each. In the first part histologic study diagnosed 64 percent as granulomas, 26 percent as cysts and 10 percent as other lesions. In the second part of the study, all thought to be cysts clinically, 84 percent were diagnosed as granulomas, 14 percent as cysts and 2 percent were other lesions.

Patterson, Shafer, and Healey⁵⁴ examined 501 biopsy specimens taken from the apical regions of endodontically involved teeth. Eighty-four percent were classified as granulomas, 14 percent as cysts and 2 percent as other pathoses.

Linenberg, Waldron and DeTowne,⁵⁵ evaluated 110 periapical specimens histologically. They found 31 were radicular cysts, 68 were granulomas, 6 were chronic periapical abscesses, and 5 were lesions of periodontitis.

Lolande and Luebke⁵⁶ conducted a study based on the microscopic examination of 800 periapical lesions. They found that periapical granulomas constituted 45.2 percent of the lesions and radicular cysts 43.8 percent. The remaining 11 percent of the lesions were related inflammatory lesions of various types.

Block, Bushell, Rodriques, and Langeland³⁷ found that there were only 14 cysts in the 230 periapical lesions in their study.

Morse, Patnik, and Schacterle,⁵⁸ studied root canal fluids that were aspirated from 40 anterior teeth with radiographic evidence of periapical pathosis, and from one pulpless anterior tooth with no radiographic

evidence of periapical pathosis. Endodontic therapy was performed on all the teeth, and all the periapical lesions were surgically removed. The root canal fluids were analyzed using polyacrylamide-gel electrophoresis. The histopathologic diagnoses of the surgical specimens were compared with the electrophoretic patterns. They found that there was an albumin pattern in all 31 of the cases that were diagnosed as granulomas and in the one pulpless anterior tooth. In eight of the nine histologically diagnosed cases of cysts, there was a larger and more intense albumin pattern, and patterns were found in the globulin regions.

CHAPTER III

METHODS AND MATERIALS

Three adult beagle type dogs of approximately two years of age were used in this study. The dogs were obtained through the Animal Research Facility (ARF) at the Loyola University Medical Center. Upon their arrival at the ARF, the dogs were observed for a minimum of two weeks to ensure good health and adaptation to a new environment. The dogs weighed between 9.2 and 10.5 Kg and were identified by round tags marked with four digit numbers 7796, 7797, 7798 respectively. These tags were tied around their necks and the numbers were thereafter recorded on all experimental data that pertained to each animal. The dogs were kept in separate cages and fed a standard diet with water ad libitum. The feeding and care of the dogs was provided by the staff at the ARF, along with veterinary consultation as needed.

PREOPERATIVE PREPARATION

Prior to the operative procedures a broth containing a known concentration of Streptococcus faecalis microorganisms was prepared in order to deliver a known number of organisms to each experimental tooth. A human isolate of S. faecalis (an enterococci; group D) maintained on a slant of Trypticase Soy agar was obtained from the Loyola University Hospital Department of Microbiology. An isolate of this culture was

transferred to a flask containing Brain-Heart Infusion (BHI) broth and incubated for eighteen hours at 37°C. Then, the optical density of 1.63 was determined using a colorimeter and a BHI broth blank. Successive dilutions of this broth culture were prepared and equal volumes from each dilution tube spread out onto trypticase soy agar plates. These plates were incubated for 24 hours at 37°C. The number of colonies per plate was determined and the dilution factor applied to determine the concentration of microorganisms in the 18 hour broth culture. A solution containing S. faecalis organisms having an optical density of 1.63 was determined to contain 2.15×10^{14} organisms per millimeter. The day before each operative procedure a flask containing BHI broth was inoculated with S. faecalis organisms and incubated for eighteen hours. The broth culture was then centrifuged at 15,000 gravities for ten minutes and the supernatant fluid discarded. The pellet was then resuspended to the original broth culture volume with saline and the procedure was repeated two more times to remove any remaining media solution. The final pellet of microorganisms was again resuspended in saline and diluted until an optical density of 1.63 was obtained. This solution was then diluted using the serial dilution method so that 538 organisms were inoculated into each operative experimental tooth.

Each dog was operated upon twice and then sacrificed. The teeth used in this study and the intervals between operations are listed in the following table (Table 1). On the day of operative procedures the operating room was prepared and the dog was anesthetized. The anesthetized dog was placed on the operating table and the maxillary and

TABLE 1

| DOG NUMBER | DAYS BETWEEN OPERATION AND SACRIFICE | TEETH USED IN STUDY |
|-----------------------|--|--|
| 7798 | 1 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \text{R} \\ \hline \text{M}_1 \text{P}_3 \text{P}_2^* \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \text{L} \\ \hline \text{P}_3 \text{P}_4 \text{M}_1^{**} \end{array}$ </div> </div> |
| 7798 | 15 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \hline \text{P}_4 \text{P}_2 \text{P}_1^* \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \hline \text{M}_1 \end{array}$ </div> </div> |
| 7796 | 30 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \hline \text{M}_1 \text{P}_4^* \text{P}_1 \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \hline 2 \text{P}_4 \text{P} \end{array}$ </div> </div> |
| 7796 | 45 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \hline \text{M}_1^{**} \text{P}_4 \text{P}_2 \text{P}_1^* \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \hline \text{P}_1 \text{M}_1 \end{array}$ </div> </div> |
| 7797 | 60 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \hline \text{P}_4 \text{P}_3^* \text{P}_2 \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \hline \text{P}_2^{**} \text{P}_3 \text{M}_1 \end{array}$ </div> </div> |
| 7797 | 90 | <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> $\begin{array}{c} \hline \text{M}_1 \text{P}_4 \text{P}_2^* \text{P}_1 \end{array}$ </div> <div style="text-align: center;"> $\begin{array}{c} \hline \text{P}_4 \end{array}$ </div> </div> |
| * operated control | | |
| ** unoperated control | | |

mandibular jaws were retracted using a spring loaded device which attached to the canine teeth.

RADIOGRAPHIC PROCEDURES

Preoperative radiographs were taken of the experimental and control teeth. The mandibular teeth were radiographed by placing the film intraorally using standard size* and pedodontic size X-ray film**. A hand held General Electric X-ray generator supplying 60 KVP at 20 ma with an exposure time of 0.2 seconds was used as the X-ray source. The maxillary teeth were X-rayed using occlusal film*** placed extraorally, the X-ray generator being aimed from the opposite side. This was necessary because the shallow palate of the dog precluded placing the film intraorally. The films were processed using routine procedures.

OPERATIVE PROCEDURES

The teeth were isolated by buccal and lingual placement of two inch by two inch sterile gauze sponges. The teeth were swabbed with a 90 per cent alcohol solution. A sterile #557 bur was placed in the high speed handpiece and the occlusal surface of the operated tooth was reduced and access was opened into the pulp chamber. The coronal pulp tissue was removed with an endodontic excavator. The access cavity was completed to facilitate placement of the S. faecalis inoculum and the access cavity

* Kodak DF58, Eastman Kodak Co., Rochester, New York

** Kodak DF54, Eastman Kodak Co., Rochester, New York

*** Kodak DF50, Eastman Kodak Co., Rochester, New York

restoration. Standard K-type endodontic files* were used to macerate the radicular pulp tissue to the depth of the apical delta. The canals were irrigated with sterile saline to remove gross blood and debris and dried with sterile cotton pellets. A dry sterile cotton pellet was placed in the pulp chamber and the previously prepared saline and S. faecalis solution were placed in the pulp chamber in a measured amount with a pipette. Approximately 538 S. faecalis microorganisms were placed in each experimental tooth. The access cavity was restored by first placing I R M** as a base. When the I R M hardened an occlusal silver amalgam*** restoration was placed to seal the access cavity. The identical procedures were performed on all experimental teeth with the exception that S. faecalis was not placed in the operated control teeth. After the operations the dogs were returned to their cages at the ARF and maintained on a soft diet.

SACRIFICE PROCEDURE

One animal was sacrificed at each of the following periods after their initial operative procedure: 15 days (#7798), 45 days (#7796), and 90 days (#7797). At the time of sacrifice the dogs were prepared as they had been previously for anesthesia. An intravenous injection of five cc. of Beuthanasia-D**** was administered producing death in less than one minute. Final radiographs were taken of the experimental and control

* Union Broach Company, Inc., Long Island City, New York

** I R M, L.W. Caulk Co., Milford, Delaware

*** Tytin, S.S. White, Penwalt Dental Products Division,
Philadelphia, Pennsylvania
S.S. White Co., Great Neck, New York

**** Burns-Biotec Laboratories Division, Chromalloy Pharmaceutical, Inc.,
Oakland, California.

teeth. The animals were transferred to the necropsy room and the soft tissue was dissected from the surrounding bone of the jaws. Block sections of the maxilla and mandible were removed with an electric bone saw and immediately placed for fixation in individual pre-marked bottles containing approximately 500 cc. of ten percent neutral buffered formalin solution.

HISTOLOGICAL PREPARATION

The block sections were further reduced using a high speed hand-piece and a #557 bur, to allow more thorough fixation. Fixation continued for two weeks, with the solution changed every two days. The sections were then rinsed in running water for four hours, and placed in a solution of ten percent formic acid for decalcification. The decalcification continued for approximately two weeks until the sections were radiolucent and rubbery. The sections were further trimmed with a razor blade into blocks containing individual teeth. These blocks were rinsed in running water for six hours and placed in increasing concentrations of alcohol over a two day period. The sections were cleared in xylol and embedded in parafin. Sections were made at six micron intervals and mounted on glass slides. The mounted tissue was deparaffinated and stained with hematoxylin and eosin or Brown-Brenn stain for light microscopic examination. A histological evaluation of the periapical areas of the experimental and control teeth was conducted.

CHAPTER IV

RESULTS

All the animals remained in good physical condition throughout the experiment. One of the dogs (7798) experienced a temporary loss of weight after the second operative period but recovered quickly and remained healthy until sacrifice.

Dog Number 7798

This dog was a male and weighed 9.8 kg. The experimental periods were one and fifteen days. The maxillary left first molar was the unoperated control for both experimental periods. The mandibular right second premolar was the operated control for the one day experimental period and the maxillary right first premolar was the operated control for the 15 day experimental period. The experimental teeth for the one day period were maxillary left third premolar, maxillary left fourth premolar, mandibular right third premolar, and mandibular right first molar. The 15 day experimental teeth were maxillary right fourth premolar, maxillary right third premolar, and mandibular left first molar.

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Radiolucencies were found in the apical areas of the maxillary right fourth premolar and the mandibular left first molar (Table 2).

Histologic Findings

The unoperated control tooth did not show any inflammatory changes in the periapical region. The one day and fifteen day operated control teeth exhibited minimal inflammatory changes in the periapical tissue. The maxillary left third and fourth premolars and the maxillary right fourth premolars showed minimal inflammatory changes in the periapical tissues. The mandibular left first molar showed mild inflammatory changes (Table 2).

Bacteriologic Findings

One section of every 30 was stained with Brown-Brenn stain. Bacteria were seen in only one tooth, the maxillary right second premolar (Table 2).

Dog Number 7796

This dog was a male and weighed 10.5 kg. The experimental periods were 30 and 45 days. The maxillary right first molar was the unoperated control for both experimental periods. The mandibular right fourth premolar was the operated control for the 30 day experimental period and the maxillary right first premolar was the operated control for the 45 day experimental period. The experimental teeth for the 30 day period were mandibular right first premolar, mandibular right first molar, maxillary left second premolar, and maxillary left fourth premolar. The experimental teeth for the 45 day experimental period were the maxillary right second premolar, maxillary right fourth premolar, mandibular left first premolar, and mandibular left first molar.

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Radiolucencies were seen in the apical areas of the mandibular right fourth premolar, mandibular right first molar, maxillary left fourth premolar, and mandibular left first molar (Table 2).

Histologic Findings

The unoperated control did not show any inflammatory changes in the periapical tissues. The 30 day operated control tooth exhibited a mild inflammatory reaction in the periapical tissue. The 45 day operated control exhibited a minimal inflammatory reaction in the periapical tissue. The maxillary left second premolar, maxillary left fourth premolar, maxillary right second premolar, and mandibular left first premolar exhibited minimal inflammatory reactions. The mandibular right first molar and maxillary right fourth premolar had a mild periapical inflammatory response. The mandibular left first molar had a moderate periapical reaction (Table 2).

Bacteriologic Findings

One section of every 30 was stained with Brown-Brenn stain. Bacteria were seen in the periapical areas of the maxillary right second premolar, maxillary left fourth premolar and the mandibular left first premolar (Table 2).

Dog Number 7797

This dog was a male and weighed 9.2 kg. The experimental periods were 60 days and 90 days. The mandibular left second premolar was the unoperated control for both time periods. The operated control for the 60 day period was the maxillary right third premolar. The operated control for the 90 day period was the mandibular right second premolar. The

experimental teeth for the 60 day period were maxillary right third premolar, maxillary right fourth premolar, mandibular left third premolar, and mandibular left first molar. The experimental teeth for the 90 day period were the mandibular right first premolar, mandibular right fourth premolar, mandibular right first molar, and maxillary left fourth premolar.

Clinical Findings

There was no clinical evidence of periapical pathosis.

Radiographic Findings

Radiolucencies were seen in the periapical areas of the maxillary right fourth premolar, mandibular left third premolar, mandibular left first molar, maxillary left third premolar, mandibular right fourth premolar, and mandibular right first molar (Table 2).

Histologic Findings

The unoperated control tooth did not exhibit any inflammatory changes in the periapical tissues. The operated control teeth for both the 60 and 90 day control periods showed moderate inflammatory responses in the periapical tissues. The mandibular right first premolar exhibited a minimal inflammatory reaction. The maxillary right fourth premolar had a mild inflammatory response. Moderate inflammatory reactions in the periapical tissues occurred in the mandibular left third premolar, mandibular left first molar, and maxillary left fourth premolar. Severe reactions were seen in the periapical tissues of the mandibular right fourth premolar and mandibular right first molar (Table 2).

Bacteriologic Findings

One section of every 30 was stained with Brown-Brenn stain. Bacteria

were seen in the periapical tissues of one tooth, the mandibular left first molar (Table 2).

TABLE 2

| Dog Number | Quadrant and Tooth | Days Operation to Sacrifice | Periapical Reaction* | | | Other Findings** |
|-----------------------|--------------------|-----------------------------|----------------------|------------------|--------------|----------------------------|
| | | | Control | Operated Control | Experimental | |
| 7798 | UL-M1 | 1-15 | 0 | | | |
| | LR-P2 | 1 | | 1 | | |
| | LR-P4 | 1 | | | 0 | |
| | LR-M1 | 1 | | | 0 | |
| | UL-P3 | 1 | | | 1 | |
| | UL-P4 | 1 | | | 1 | |
| 7798 | UR-P1 | 15 | | 1 | | |
| | UR-P4 | 15 | | | 1 | PR |
| | UR-P2 | 15 | | | 0 | BA |
| | LL-M1 | 15 | | | 2 | PR |
| 7796 | UR-M1 | 30-45 | 0 | | | |
| | LR-P4 | 30 | | 2 | | PR |
| | LR-P1 | 30 | | | 0 | |
| | LR-M1 | 30 | | | 2 | PR |
| | UL-P2 | 30 | | | 1 | BA |
| | UL-P4 | 30 | | | 1 | PR BA |
| 7796 | UR-P1 | 45 | | 1 | | |
| | UR-P2 | 45 | | | 1 | |
| | UR-P4 | 45 | | | 2 | |
| | LL-P1 | 45 | | | 1 | BA |
| | LL-M1 | 45 | | | 3 | PR |
| 7797 | LL-P2 | 60-90 | 0 | | | |
| | UR-P3 | 60 | | 3 | | |
| | UR-P4 | 60 | | | 2 | PR |
| | LL-M1 | 60 | | | 3 | PR BA |
| | LL-P3 | 60 | | | 3 | PR |
| 7797 | LR-P2 | 90 | | 3 | | PR |
| | LR-P1 | 90 | | | 1 | |
| | LR-P4 | 90 | | | 4 | PR |
| | LR-M1 | 90 | | | 4 | PR |
| | UL-P4 | 90 | | | 3 | |
| * Periapical Reaction | | 2 | Mild Reaction | | | ** other findings |
| 0 | No Reaction | 3 | Moderate Reaction | | | PR Periapical Radiolucency |
| 1 | Minimal Reaction | 4 | Severe Reaction | | | BA Bacteria Present |

CHAPTER V

DISCUSSION

The purpose of this study was to ascertain whether predictable pulpo-periapical pathosis could be produced in dog teeth using a method of removing the coronal pulp, macerating the contents of the pulp canal with an endodontic file, and then inoculating the pulp chamber with a suspension of a known number of S. faecalis microorganisms. In performing endodontic research where periapical healing is to be evaluated, it is necessary first to have an area of pathosis from which a judgement of the healing potential of the treatment can be made. Therefore it is highly desirable to have a method of produce predictable periapical lesions even if the pathosis is not yet radiographically evident. Bender and Seltzer,⁵⁸ have shown that periapical lesions may be present but not seen in a radiograph unless there is perforation of the bone cortex, erosion from the inner surface of the bone cortex, or extensive erosion or destruction from the outer surface. They contend that lesions which are present only in cancellous bone cannot be detected radiographically. Also Fontain,⁵⁹ has stated that bone must have a 30-60% reduction in calcification before showing radiographic changes.

All the teeth from the period of 45 days or longer including both the operated controls and those inoculated with S. faecalis, exhibited histologically verifiable periapical lesions. However, of those 14 teeth,

only 7 (50.0%) had accompanying periapical lesions on radiographs.

In general, the teeth which demonstrated the more severe periapical reactions according to histologic evaluation, corresponded to those exhibiting apparent radiographic lesions as well (6 out of 8, 75%).

A trend indicated that the longer the time from operation to sacrifice, the more severe was the inflammatory reaction. This was true of those teeth which were inoculated with S. faecalis and those teeth which were operated but uninoculated controls. It should be noted, however, that the most severe inflammatory reactions occurred only in the inoculated teeth. This observation gives credence to the widely held desire to eliminate bacterial contamination in endodontic procedures. The combination of instrumentation and bacteria gave a more severe reaction than instrumentation alone.

All mandibular teeth with moderate and severe histologic reactions had radiolucencies. The maxillary teeth with similar pathosis did not have detectable radiographic changes. The reason for this may be that the maxillary sinus is very close to the apices of the posterior teeth in the dog, and it may be more difficult to see radiolucencies in the maxilla as compared to the mandible. Also, since the dog has a flat palate, it was necessary to place the X-ray film extraorally for the maxillary teeth, and direct the X-ray beam at an angle from the opposite side. This further obscured the roots of the maxillary teeth because of the projection of the maxillary sinus over the apices. If definable X-ray lesions are needed for a research project, it would be better to use only mandibular teeth.

All teeth operated upon had an occlusal seal that consisted of a zinc oxide-type cement base and a silver amalgam filling. Regardless of the adequate coronal seal, periapical inflammatory reactions developed in 24 of 28 teeth including the operated upon, but uninfected, control teeth. Furthermore, all of the teeth without inflammatory reactions were from the shortest experimental periods. Two of the four were from the one day period, one was from the 15 day period, and the remaining tooth was from the 30 day period. This finding is in contrast to a study by Sinai, et al.,⁶⁰ who performed partial pulp extirpations in 24 Rhesus monkey teeth. Histologic evaluation was then made of the remaining apical pulp stump and periapical tissues from immediately to six months after extirpation. In that study, for a period up to one month an acute inflammatory response developed in the remaining apical pulp stump and periapical periodontal ligament. This reaction, however, was followed by repair.

In this study, occlusal seal did not produce healing or even cessation of destruction. The lesions continued to increase in size and severity as time went on. The concept of occlusal seal gaining periapical healing was shocking to the endodontic community when originally published. On the basis of this study, its acceptance is severely questioned.

Bacteria were observed in the periapical tissues adjacent to the canal foramina in five of the teeth, all of which were inoculated with S. faecalis. This occurred only in the 15, 45 and 60 day periods, but not in the one day or 90 day periods. It could be speculated that it took a minimum of 15 days for the S. faecalis to become established in

the periapical tissues, but after 90 days the inflammatory response at the periapex was sufficient to eliminate the microorganisms. The finding of microorganisms in the periapical tissues is supported by the findings of Winkler and associates,⁶¹ who examined 15 extracted teeth with attached periapical lesions. After finding these lesions to be clinically and histologically intact, they sectioned and stained the lesions and found bacteria to be uniformly distributed throughout the lesion. Block, et al.,³⁷ however, studied 230 periapical endodontic surgical specimens and found that bacteria occurred very infrequently in the periapical tissues.

One of the problems that confronts an investigator conducting a research project is the accurate evaluation of the results. Bias is impossible to eliminate even though a diligent attempt is made to be objective. This research was carried out by one investigator, who, although consulting with others, did all the evaluation himself. A conscientious effort was made to avoid bias but the inherent weakness of this approach should be recognized.

A scale of 0 to 4 was used to grade the degree of the inflammatory reaction. This corresponded to an inflammatory response, respectively of, none, minimal, mild, moderate, and severe. There were no clear-cut boundaries between these categories. The microscopic slides of the tissue specimens were compared and ranked on a relative basis. The specimens that had no inflammation were ranked 0. Ranking the specimens with inflammation into separate categories was more difficult and required a considerable amount of time.

Another problem that complicated the evaluation of the tissue

specimens was that the cell nuclei did not stain well with hematoxlin. One reason for this problem was a possible faulty decalcification technique. There are several methods to decalcify hard tissues before sectioning and staining. One optimal technique is to use a weak organic acid and perform the decalcification over a long period of time. Due to time restraints, however, it was necessary to perform decalcification more rapidly. A 10% formic acid solution was used, which is not excessively strong, but it may have, nevertheless, damaged the specimens so they did not stain properly. Another reason for the faulty staining could lie with the method of fixation used. After the animals were sacrificed, the jaws containing the teeth used in the study were block-resected and placed in 10% formalin solution. A time lapse of up to one hour may have occurred from the time of death to placement of the resected jaw in the formalin. In this period of time, damage could possibly have occurred to the tissues that prevented proper uptake of stain. Also, it may be possible that since smaller sections of the individual teeth were not made until the next day, that the 10% formalin solution did not penetrate the initial large block sections. In retrospect, better results would probably have resulted if the dog was perfused with a fixative at the time of sacrifice.

Regardless of this problem, inflammatory cells were easily seen in the microscopic sections, but it was not always possible to identify the individual cell types that are normally seen in periapical inflammatory responses such as polymorphonuclear neutrophils, lymphocytes, monocytes, plasma cells, foam cells, macrophages, mast cells and foreign body giant cells.

plasma cells, foam cells, macrophages, mast cells and foreign body giant cells.

The operative procedures performed on the dogs were done at the Animal Research Facility (ARF) at the Loyola University Medical Center. The personnel at the ARF were very cooperative in scheduling the operating rooms and were in charge of the day to day care of the dogs. They also administered the anesthetics at each operative session and the lethal injections at the time of sacrifice. During the actual operative procedures the work was done alone, but it would have had been very advantageous to have had an assistant. It was especially difficult to x-ray the dogs unassisted as the procedures required positioning the dog, placing the film, and then stabilizing the dog and film while using both hands to hold and operate the portable x-ray unit. An assistant would have also been very helpful during the endodontic and inoculating procedures.

The dogs used in this study were easy to handle and operate upon. Anesthesia was administered without difficulty using a vein in the foreleg as an intravenous route. The dogs recovered uneventfully from the anesthetic and the operative procedures.

A spring-loaded mouth prop was used to separate the dogs jaws in order to gain good access to the teeth for the endodontic procedures (see figure 3). A rubber dam could have been used to isolate the teeth but this was not necessary because atropine was administered which dried the mouth sufficiently so that the teeth could be adequately isolated with sterile 2 x 2 gauze pads (see figure 5).

The pulp chamber of the dog tooth is large and easily located and instrumented. Each root canal is well separated and could be used for different experimental procedures if desired. The pulp tissue of the dog is morphologically similar to that of the human,¹ but the apical area is quite different in that the main canal usually breaks up into a complexity of channels that radiate peripherally. Because of this difference, and since apical structures are so important in influencing the spread of inflammation from the pulp to the periapical area, the dog may be a poor model for human endodontic research.

SUMMARY

1. Histologically verifiable periapical lesions can be produced in 45 days in dog teeth using the method of removing the coronal pulp, macerating the contents of the root canal with an endodontic file, and inoculating the canal with a suspension of 528 S. faecalis microorganisms.
2. The inflammatory reaction became more severe with time.
3. Intact occlusal seal did not prevent the development of periapical pathosis.
4. Periapical radiolucencies developed in all mandibular teeth with moderate to severe periapical inflammatory reactions.
5. Periapical radiolucencies were more easily detected in the mandible of the dog.
6. Bacteria are sometimes found in the periapical tissues of dog teeth infected with S. faecalis, but not before 15 days or after 90 days.
7. The most severe periapical reactions occurred only in the inoculated teeth.

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FIGURES

Figure 1: Instruments used for endodontic procedures on dogs.

Figure 2: X-ray film positioned intraorally for X-ray of mandibular teeth.



Figure 3: X-ray in position extraorally for radiographs of maxillary teeth.

Figure 4: Hand held X-ray unit used for radiographs.



Figure 5: Access cavity in mandibular third premolar. Number 20 root canal file in distal canal.

Figure 6: Suspension of S. faecalis placed in pulp chamber and covered with a sterile cotton pellet.



Figure 7: Cotton pellet covered with cement base.

Figure 8: Final silver amalgam restoration.



Figure 9: Preoperative view, 60 day experimental animal.

Figure 10: Postoperative view, 60 day experimental animal,
radiolucent area at LL M1.

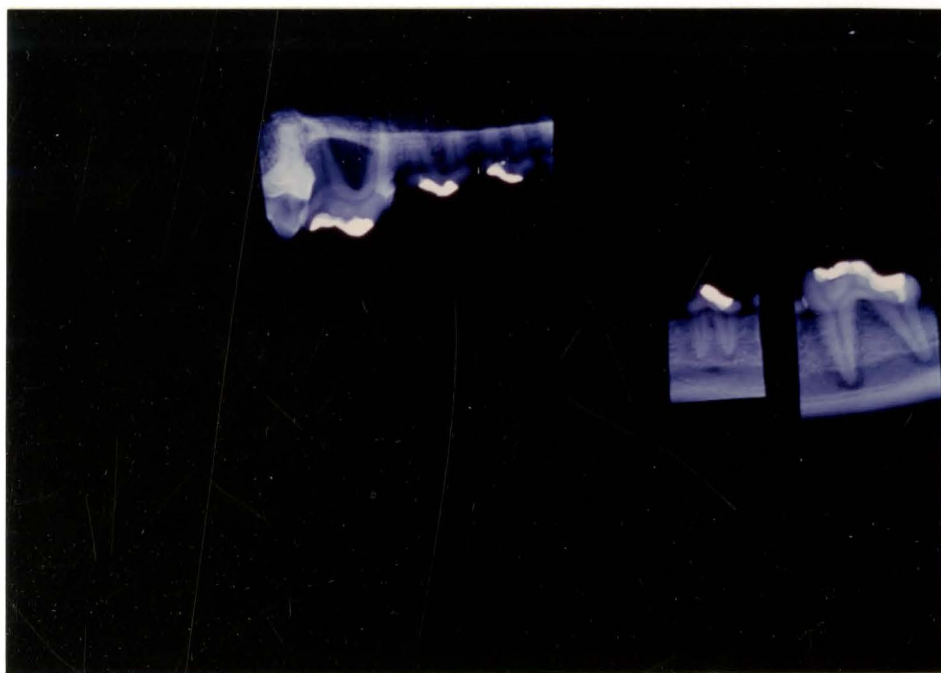
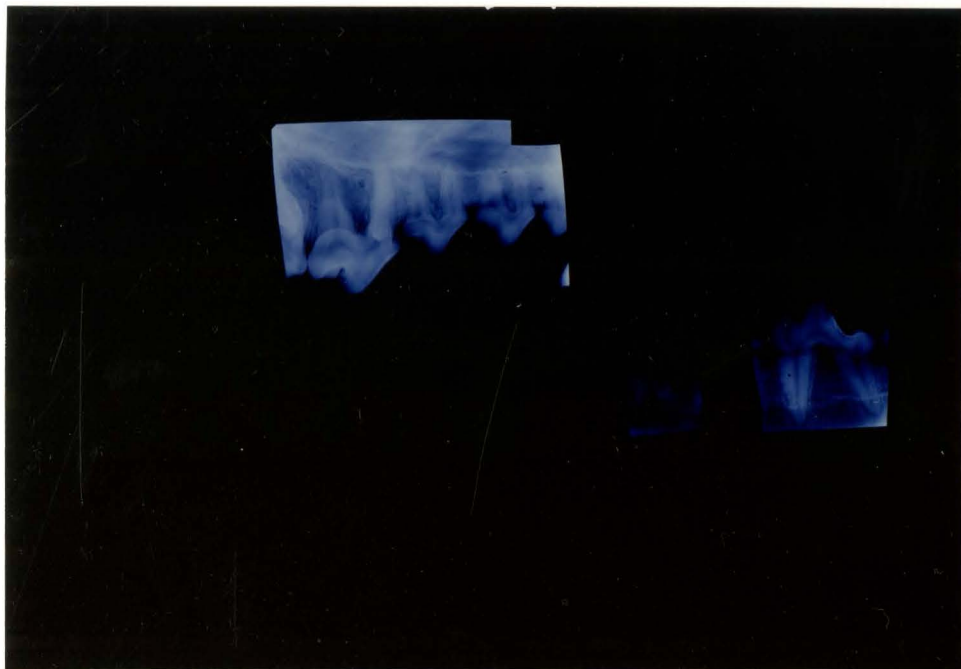


Figure 11: Preoperative view, 90 day experimental animal.

Figure 12: Postoperative view, 90 day experimental animal, periapical radiolucencies present LR M1, P3, P4.

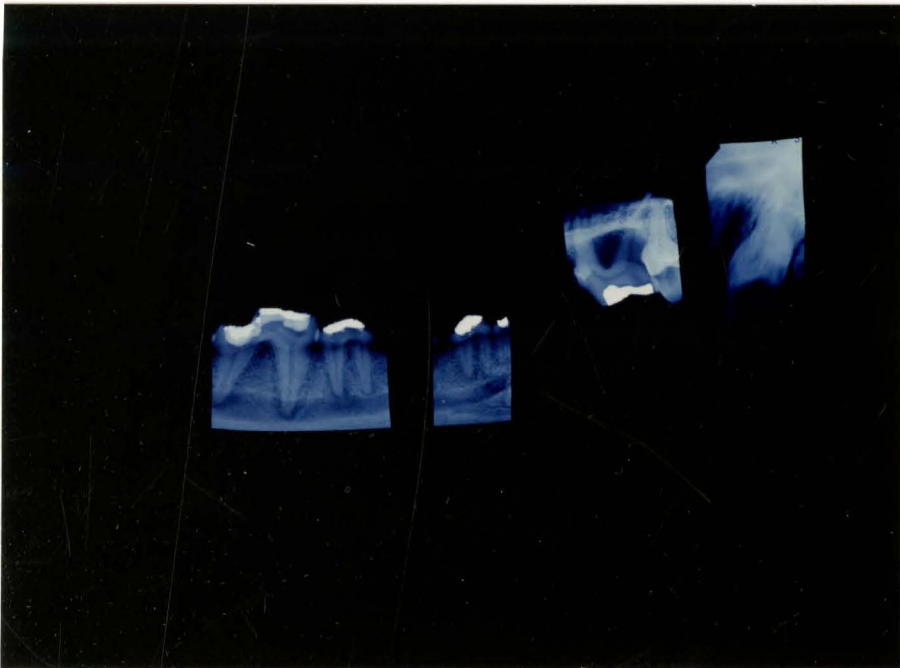
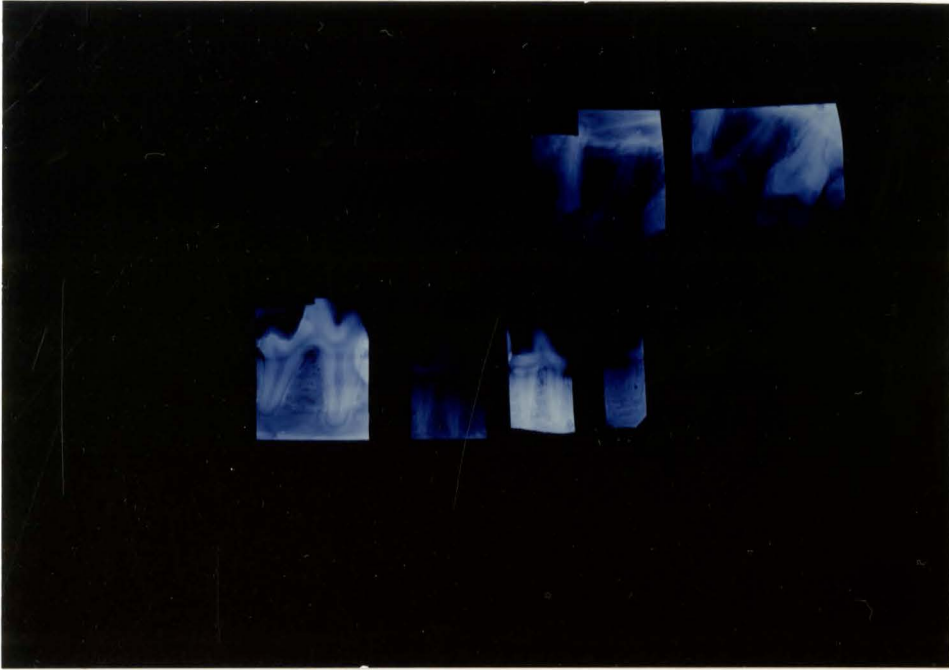


Figure 13: Apex of unoperated control tooth (one day sacrifice). Normal apical tissues. Apical foramina (A), periodontal ligament (P), alveolar bone (B). (H&E stain, X40)

Figure 14: Minimal inflammatory reaction (15 d. exp.). Cementum (C), minimal inflammatory exudate (I). Portion of apex removed in sectioning. (H&E stain, X40)

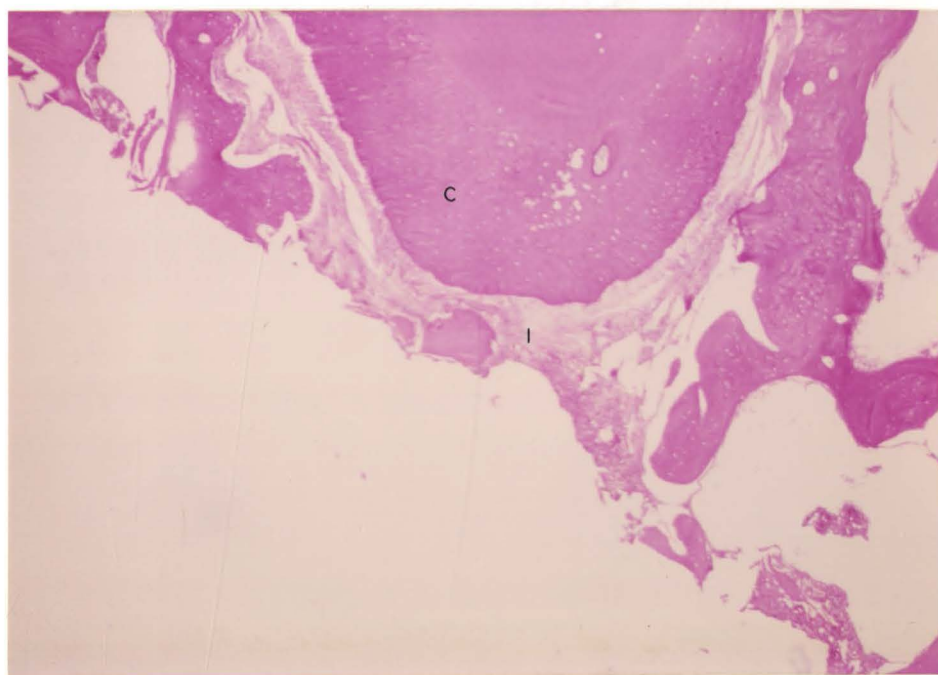
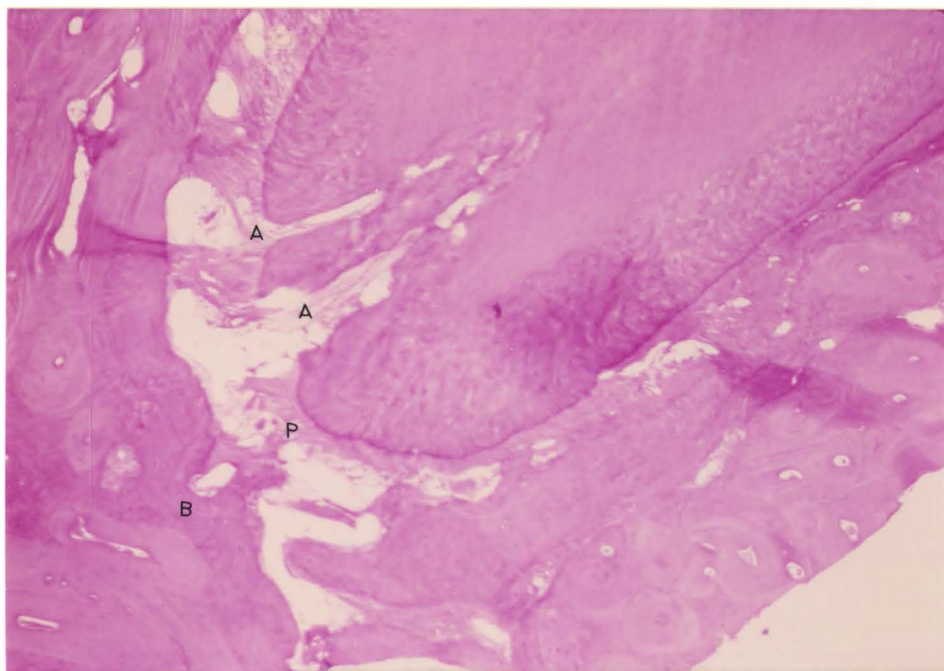


Figure 15: Mild inflammatory reaction (15 d exp.). Mild inflammatory exudate (I), apical canals (A). (H&E stain, X40)

Figure 16: Moderate inflammatory reaction (60 d exp.). (H&E stain, X40)

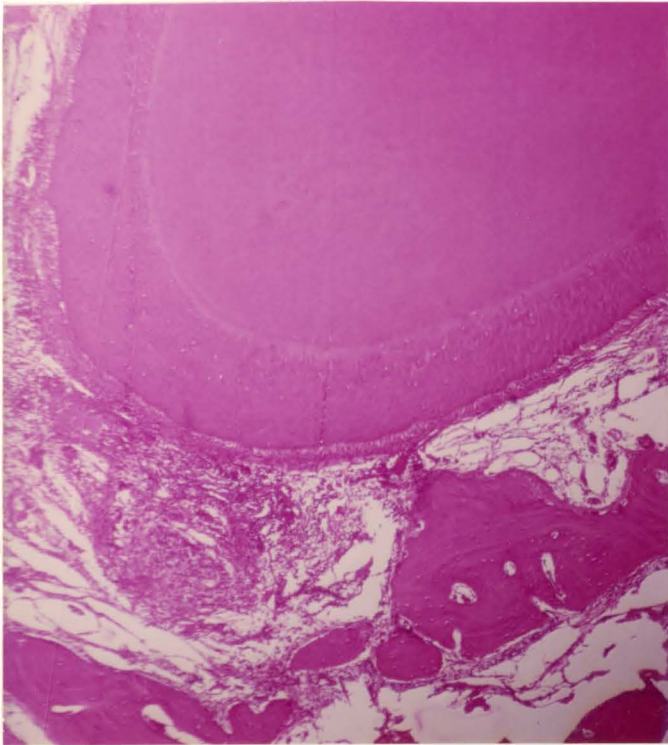
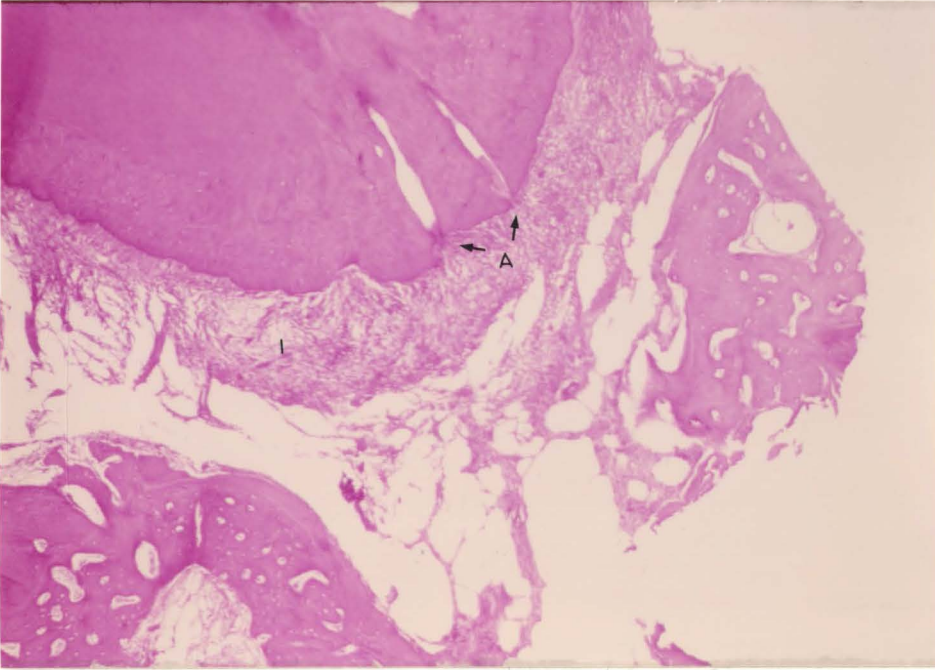


Figure 17: Ninety day experimental animal . Note abundant fibrous tissue (F), and minimal number of inflammatory cells. This might appear to be spontaneous healing. Blood vessels (V), bone resorption (arrow). (H&E stain, X40)

Figure 18: Box area of figure 17 enlarged to 100X. Note minimum of inflammatory exudate but abundant fibrous tissue (F), blood vessels (V).

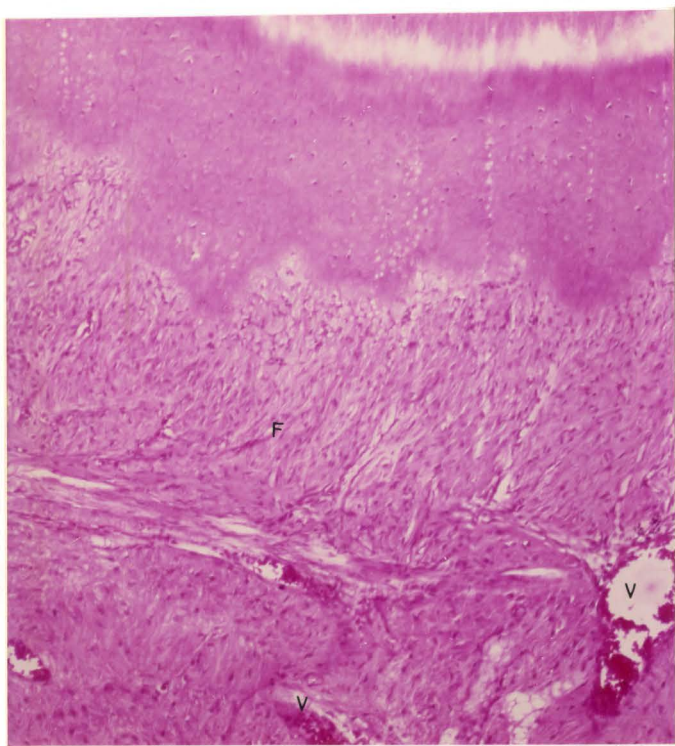
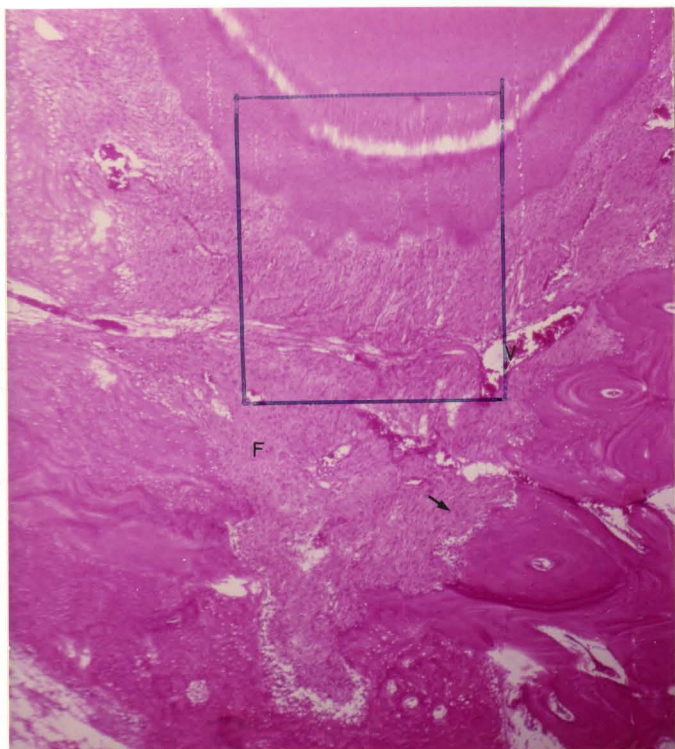


Figure 19: Severe inflammatory reaction (90 d exp.) dentin (D), cementum (C), inflammatory exudate (I), alveolar bone (B). (H&E stain, X100)

Figure 20: Box area of figure 19 enlarged to 400X. Note chronic inflammatory cells (C), blood vessels (V), and fibroblasts (F), typical of granulation tissue.

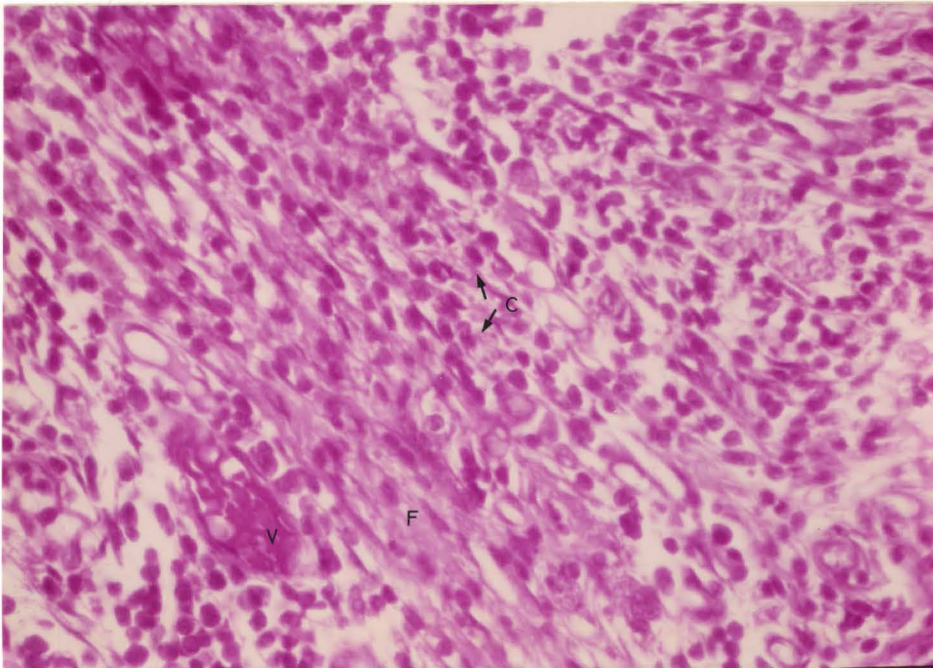
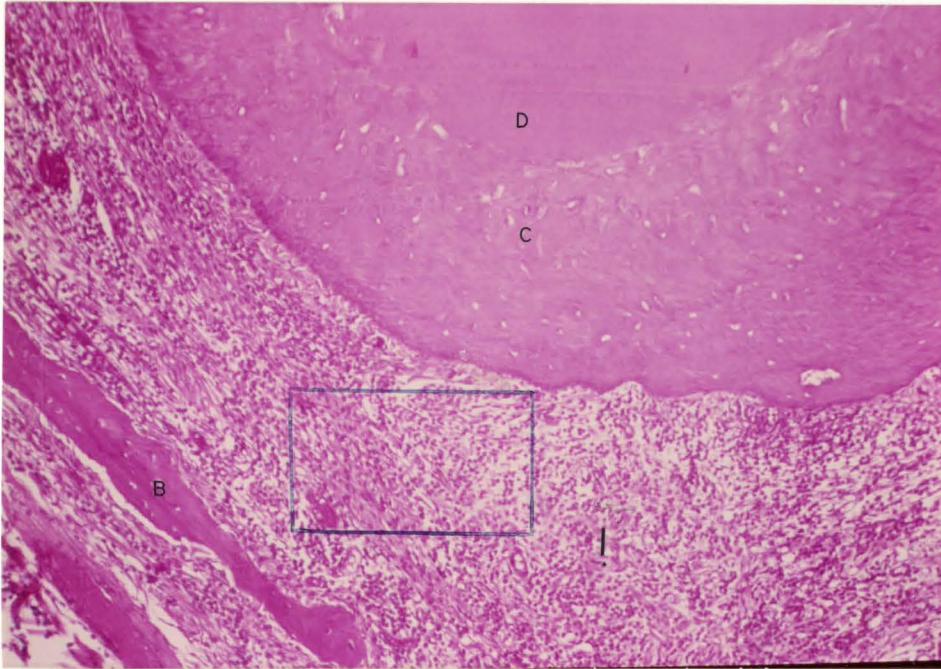


Figure 21: Area of hypercementosis (H), surrounded by moderate inflammatory exudate (I) (90 d. exp.) (H&E stain, X40)

Figure 22: Severe inflammatory reaction (90 d. exp.) Note dense inflammatory exudate (I). (H&E stain, X40)

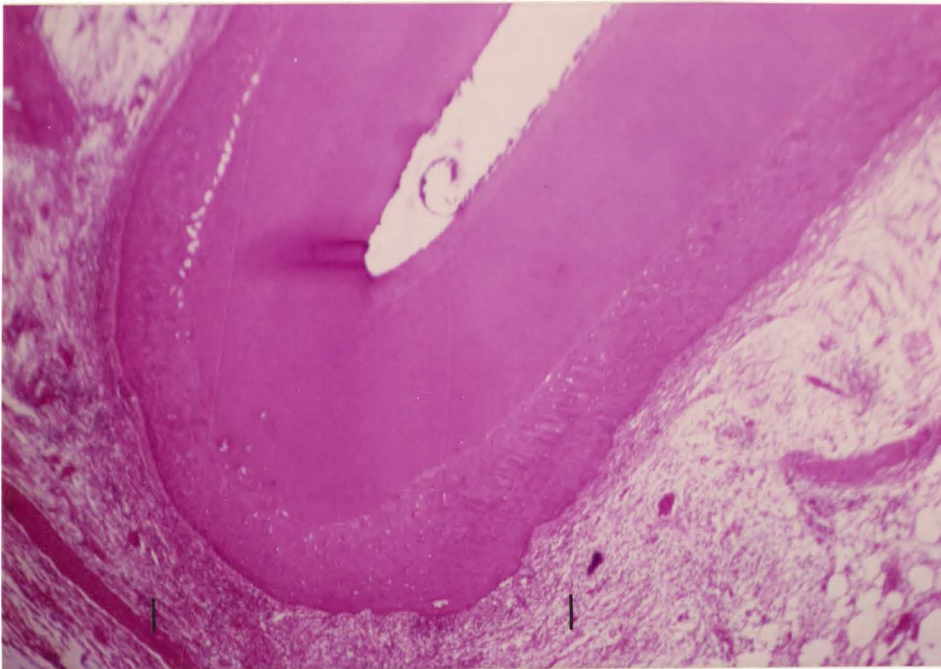
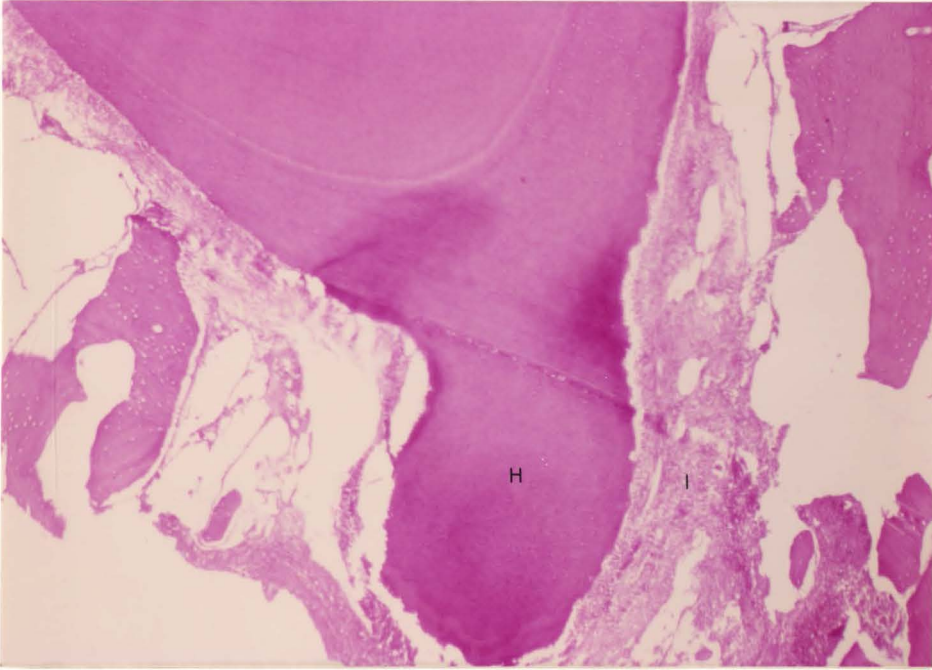
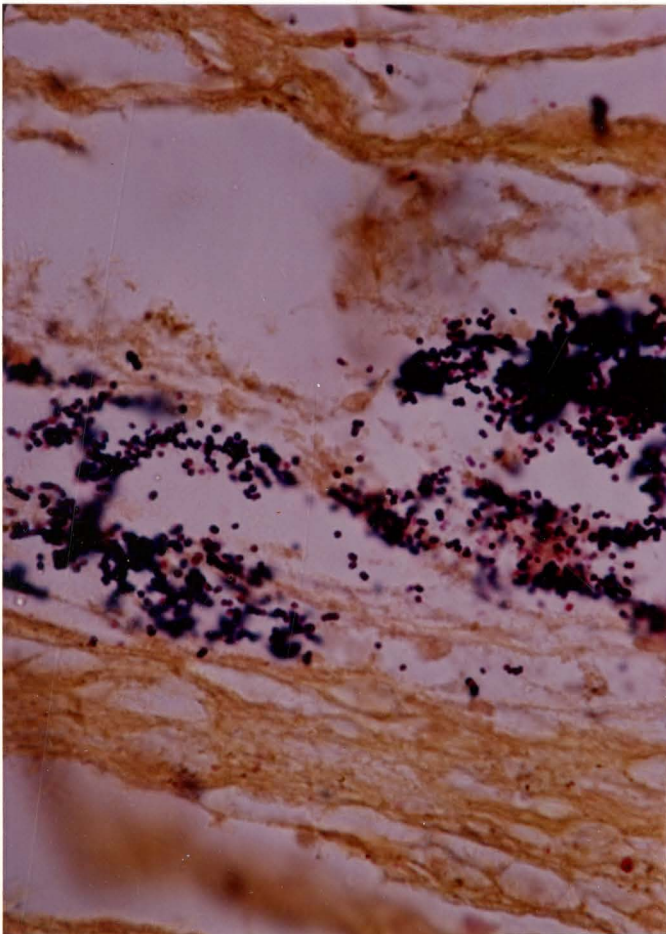
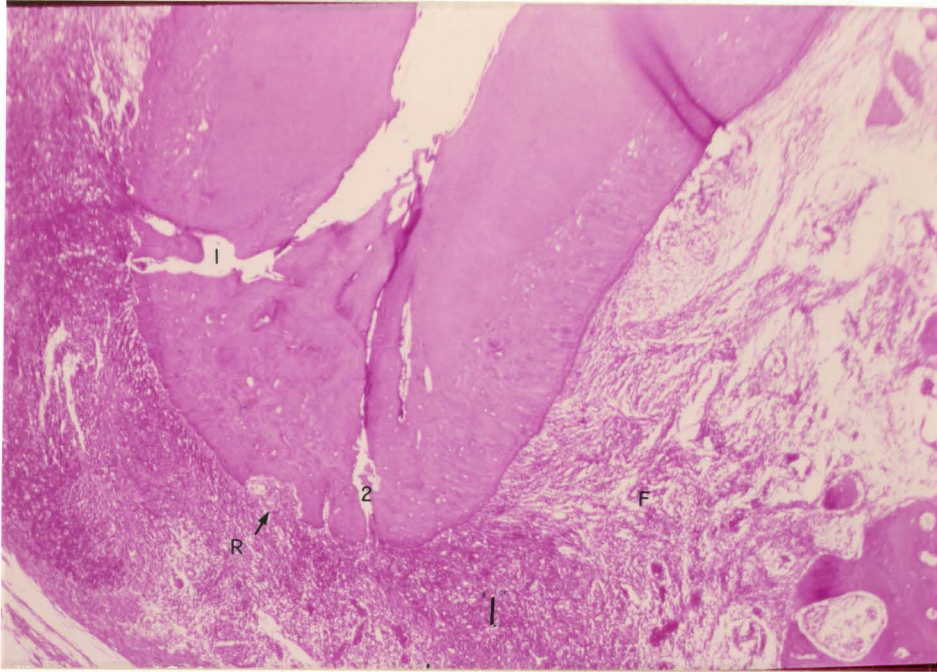


Figure 23: Apical area showing main canal (1), accessory canal (2), severe inflammatory reaction (I), cementum resorption (R), fibrous tissue at periphery of inflammatory exudate (F). (90 d. exp.) (H&E stain, X40)

Figure 24: Bacteria in periapical tissue (B) (60 d. exp.) (Brown-Brenn stain, X1000)



APPROVAL SHEET

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the above committee with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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April 12, 1984

Date



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